East

L Number	Hits	Search Text	DB	Time stamp
1	25	Wong near Grace.in.	USPAT; US-PGPUB; EPO; DERWENT	2002/06/06 13:27
6	10	Wong near Grace.in. and TNF	USPAT; US-PGPUB; EPO; DERWENT	2002/06/06 13:28
11	1282	arachidonate 12-lipoxygenase	USPAT; US-PGPUB; EPO; DERWENT	2002/06/06 13:28
16	14	arachidonate adj1 12-lipoxygenase	USPAT; US-PGPUB; EPO; DERWENT	2002/06/06 13:32
21	11	arachidonate adj1 12-lipoxygenase and TNF	USPAT; US-PGPUB; EPO; DERWENT	2002/06/06 13:33
26	5	TNF and IFN adj1 stimulated adj1 gene?	USPAT; US-PGPUB; EPO; DERWENT	2002/06/06 13:34

	U	1	~~Document ID~~	Issue Date	Pages	Title	Current OR
1	☒	☐	US 4963368 A	19901016	8	Oxidoreductase enzyme stabilized highly unsaturated fatty acids and derivatives of such acids	424/498
2	☒	☐	US 5578444 A	19961126	264	Sequence-directed DNA-binding molecules compositions and methods	435/6
3	☒	☐	US 5641755 A	19970624	23	Regulation of x-ray mediated gene expression	514/44
4	☒	☐	US 5693463 A	19971202	87	Method of ordering sequence binding preferences of a DNA-binding molecule	435/6
5	☒	☐	US 5716780 A	19980210	86	Method of constructing sequence-specific DNA-binding molecules	435/6
6	☐	☐	US 5726014 A	19980310	262	Screening assay for the detection of DNA-binding molecules	435/6
7	☒	☐	US 5738990 A	19980414	94	Sequence-directed DNA-binding molecules compositions and methods	435/6
8	☒	☐	US 5744131 A	19980428	95	Sequence-directed DNA-binding molecules compositions and methods	424/78.08
9	☒	☐	US 5869241 A	19990209	270	Method of determining DNA sequence preference of a DNA-binding molecule	435/6
10	☒	☐	US 6010849 A	20000104	270	Sequence-directed DNA binding molecules compositions and methods	435/6
11	☒	☐	US 6017536 A	20000125	611	Simian immunodeficiency virus peptides with antifusogenic and antiviral activities	424/188.1
12	☒	☐	US 6025194 A	20000215	62	Nucleic acid sequence of senescence asssociated gene	435/320.1

	Current XRef	Retrieval Classif	Inventor	S	C	P	2	3	4	5
1	424/490; 424/94.2; 424/94.4		Antrim, Richard L. et al.	☐	☐	☐	☐	☐	☐	☐
2	435/7.23; 536/23.1		Edwards, Cynthia A. et al.	☐	☐	☐	☐	☐	☐	☐
3	424/9.2; 435/29; 435/6; 514/396; 536/24.1		Weichselbaum, Ralph R. et al.	☐	☐	☐	☐	☐	☐	☐
4	435/7.23; 536/23.1		Edwards, Cynthia A. et al.	☐	☐	☐	☐	☐	☐	☐
5	436/501		Edwards, Cynthia A. et al.	☐	☐	☐	☐	☐	☐	☐
6	435/91.2; 436/501		Edwards, Cynthia A. et al.	☒	☐	☐	☐	☐	☐	☐
7	435/320.1; 435/69.1; 536/24.1		Edwards, Cynthia A. et al.	☐	☐	☐	☐	☐	☐	☐
8	436/501; 514/1		Edwards, Cynthia A. et al.	☐	☐	☐	☐	☐	☐	☐
9	435/91.1; 435/91.2		Edwards, Cynthia A. et al.	☐	☐	☐	☐	☐	☐	☐
10	435/7.1		Edwards, Cynthia A. et al.	☐	☐	☐	☐	☐	☐	☐
11	424/208.1; 530/300; 530/324; 530/325; 530/326		Barney, Shawn O'Lin et al.	☐	☐	☐	☐	☐	☐	☐
12	435/325; 536/23.1; 536/23.5; 536/24.1		Funk, Walter	☐	☐	☐	☐	☐	☐	☐

	Image Doc. Displayed	PT
1	US 4963368	☐
2	US 5578444	☐
3	US 5641755	☐
4	US 5693463	☐
5	US 5716780	☐
6	US 5726014	☐
7	US 5738990	☐
8	US 5744131	☐
9	US 5869241	☐
10	US 6010849	☐
11	US 6017536	☐
12	US 6025194	☐

	U	1	~~Document ID~~	Issue Date	Pages	Title	Current OR
13	☒	☐	US 6228983 B1	20010508	722	Human respiratory syncytial virus peptides with antifusogenic and antiviral activities	530/300
14	☐	☐	US 6384208 B1	20020507	98	Sequence directed DNA binding molecules compositions and methods	536/24.1

	Current XRef	Retrieval Classif	Inventor	S	C	P	2	3	4	5
13	424/186.1; 424/211.1; 530/324; 530/325; 530/326		Barney, Shawn O'Lin et al.	☐	☐	☐	☐	☐	☐	☐
14	536/23.1		Edwards, Cynthia A. et al.	☒	☐	☐	☐	☐	☐	☐

	Image Doc. Displayed	PT
13	US 6228983	☐
14	US 6384208	☐

	U	1	~~Document ID~~	Issue Date	Pages	Title	Current OR
1	☐	☐	US 20010031859 A1	20011018	27	High level cytokine production with enhanced cell viability	530/351
2	☐	☐	US 5976800 A	19991102	32	Enhancement of cancer cell death	435/6
3	☐	☐	US 6200780 B1	20010313	52	Human interferon-.epsilon.(IFN-.epsilon.), a type I interferon	435/69.51
4	☒	☐	US 6299869 B1	20011009	50	Human interferon-epsilon: a type I interferon	424/85.4
5	☐	☐	US 6331396 B1	20011218	87	Arrays for identifying agents which mimic or inhibit the activity of interferons	435/6

	Current XRef	Retrieval Classif	Inventor	S	C	P	2	3	4	5
1	435/366; 435/69.5		Lau, Allan S. et al.	☒	☐	☐	☐	☐	☐	☐
2	435/15		Lau, Allan S. et al.	☒	☐	☐	☐	☐	☐	☐
3	424/85.4; 435/252.3; 435/252.33; 435/254.2; 435/320.1; 435/325; 435/358; 530/351; 536/23.52		Chen, Jian et al.	☒	☐	☐	☐	☐	☐	☐
4	435/252.33; 435/320.1; 435/325; 435/358; 435/69.1; 435/69.2; 435/69.51; 514/12; 514/2; 530/350; 530/351; 536/23.1; 536/23.52		Chen, Jian et al.	☐	☐	☐	☐	☐	☐	☐
5	435/287.2; 536/23.1; 536/23.52; 536/24.3; 536/24.31		Silverman, Robert H. et al.	☒	☐	☐	☐	☐	☐	☐

	Image Doc. Displayed	PT
1	US 20010031859	☐
2	US 5976800	☐
3	US 6200780	☐
4	US 6299869	☐
5	US 6331396	☐

FILE 'HOME' ENTERED AT 13:12:19 06 JUN 2002

=> file medline
COST IN U.S. DOLLARS STN

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 13:12:28 ON 06 JUN 2002

FILE LAST UPDATED: 5 JUN 2002 (20020605/UP). FILE COVERS 1958 TO DATE.

On April 22, 2001, MEDLINE was reloaded. See HELP RLOAD for details.

MEDLINE now contains IN-PROCESS records. See HELP CONTENT for details.

MEDLINE is now updated 4 times per week. A new current-awareness alert frequency (EVERYUPDATE) is available. See HELP UPDATE for more information.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2001 vocabulary. Enter HELP THESAURUS for details.

The OLDMEDLINE file segment now contains data from 1958 through 1965. Enter HELP CONTENT for details.

Left, right, and simultaneous left and right truncation are available in the Basic Index. See HELP SFIELDS for details.

THIS FILE CONTAINS CAS REGISTRY NUMBERS FOR EASY AND ACCURATE SUBSTANCE IDENTIFICATION.

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=> s (Wong, G.? or Wong G.?)/au
           437 WONG, G.?/AU
           437 WONG G.?/AU
L1         437 (WONG, G.? OR WONG G.?)/AU

=> s TNF and IFN
         36408 TNF
            97 TNFS
         36422 TNF
                 (TNF OR TNFS)
         35502 IFN
          1488 IFNS
         35620 IFN
                 (IFN OR IFNS)
L2        7520 TNF AND IFN

=> s L1 and L2
L3           5 L1 AND L2

=> d L3 1-3

L3   ANSWER 1 OF 5     MEDLINE
AN   94317909     MEDLINE
DN   94317909   PubMed ID: 8042859
TI   Cytokine regulation of lactate dehydrogenase-elevating virus: inhibition
     of viral replication by interferon-gamma.
AU   Cafruny W A; Bradley S E; Broen J J; Wong G H
CS   Department of Microbiology, School of Medicine, University of South
     Dakota, Vermillion 57069.
NC   AI27978 (NIAID)
SO   ANTIVIRAL RESEARCH, (1994 Apr) 23 (3-4) 191-201.
     Journal code: 6I7; 8109699. ISSN: 0166-3542.
CY   Netherlands
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   199408
ED   Entered STN: 19940905
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Last Updated on STN: 19940
Entered Medline: 19940825

L3 ANSWER 2 OF 5 MEDLINE
AN 93056500 MEDLINE
DN 93056500 PubMed ID: 1331233
TI Antiviral activity of tumor necrosis factor is signaled through the 55-kDa type I **TNF** receptor [corrected].
CM Erratum in: J Immunol 1993 Jan 15;150(2):705
AU **Wong G H**; Tartaglia L A; Lee M S; Goeddel D V
CS Department of Immunology, Genentech, Inc., South San Francisco, CA 94080.
SO JOURNAL OF IMMUNOLOGY, (1992 Nov 15) 149 (10) 3350-3.
Journal code: IFB; 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199212
ED Entered STN: 19930122
Last Updated on STN: 19970203
Entered Medline: 19921208

L3 ANSWER 3 OF 5 MEDLINE
AN 92110786 MEDLINE
DN 92110786 PubMed ID: 1764697
TI Augmentation of cytotoxicity using combinations of interferons (types I and II), tumor necrosis factor-alpha, and tamoxifen in MCF-7 cells.
AU Tiwari R K; **Wong G Y**; Liu J; Miller D; Osborne M P
CS Breast Cancer Research Laboratory, Memorial Sloan Kettering Cancer Center, New York, NY 10021.
NC P-01 CA 29502 (NCI)
SO CANCER LETTERS, (1991 Dec 9) 61 (1) 45-52.
Journal code: CMX; 7600053. ISSN: 0304-3835.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199202
ED Entered STN: 19920308
Last Updated on STN: 19970203
Entered Medline: 19920220

=> s arachidonate 12-lipoxygenase
7085 ARACHIDONATE
12 ARACHIDONATES
7090 ARACHIDONATE
(ARACHIDONATE OR ARACHIDONATES)
605339 12
9944 LIPOXYGENASE
1313 LIPOXYGENASES
10065 LIPOXYGENASE
(LIPOXYGENASE OR LIPOXYGENASES)
L4 454 ARACHIDONATE 12-LIPOXYGENASE
(ARACHIDONATE(W)12(W)LIPOXYGENASE)

=> s L2 and L3
L5 5 L2 AND L3

=> d L5 1-5

L5 ANSWER 1 OF 5 MEDLINE
AN 94317909 MEDLINE
DN 94317909 PubMed ID: 8042859
TI Cytokine regulation of lactate dehydrogenase-elevating virus: inhibition of viral replication by interferon-gamma.
AU Cafruny W A; Bradley S E; Broen J J; **Wong G H**
CS Department of Microbiology, School of Medicine, University of South

Dakota, Vermillion 57069.
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SO ANTIVIRAL RESEARCH, (1994 Apr) 23 (3-4) 191-201.
Journal code: 6I7; 8109699. ISSN: 0166-3542.
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EM 199408
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Last Updated on STN: 19940905
Entered Medline: 19940825

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Journal code: IFB; 2985117R. ISSN: 0022-1767.
CY United States
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EM 199212
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L5 ANSWER 3 OF 5 MEDLINE
AN 92110786 MEDLINE
DN 92110786 PubMed ID: 1764697
TI Augmentation of cytotoxicity using combinations of interferons (types I and II), tumor necrosis factor-alpha, and tamoxifen in MCF-7 cells.
AU Tiwari R K; **Wong G Y**; Liu J; Miller D; Osborne M P
CS Breast Cancer Research Laboratory, Memorial Sloan Kettering Cancer Center, New York, NY 10021.
NC P-01 CA 29502 (NCI)
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Journal code: CMX; 7600053. ISSN: 0304-3835.
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DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199202
ED Entered STN: 19920308
Last Updated on STN: 19970203
Entered Medline: 19920220

L5 ANSWER 4 OF 5 MEDLINE
AN 91176664 MEDLINE
DN 91176664 PubMed ID: 2127737
TI Constitutive production of novel macrophage-activating factor(s) by human T cell hybridomas.
AU Taniyama T; Taki S; Akiyama Y; Yoshizawa K; Hamuro J; Arai K; **Wong G G**
CS Department of Cellular Immunology, National Institute of Health, Tokyo, Japan.
SO CLINICAL AND INVESTIGATIVE MEDICINE. MEDECINE CLINIQUE ET EXPERIMENTALE, (1990 Dec) 13 (6) 305-12.
Journal code: DFG; 7804071. ISSN: 0147-958X.
CY Canada
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 199104
ED Entered STN: 19910519
Last Updated on STN: 19910519
Entered Medline: 19910426

L5 ANSWER 5 OF 5 MEDLINE
AN 87039375 MEDLINE
DN 87039375 PubMed ID: 2430188
TI Tumour necrosis factors alpha and beta inhibit virus replication and synergize with interferons.
AU **Wong G H;** Goeddel D V
SO NATURE, (1986 Oct 30-Nov 5) 323 (6091) 819-22.
Journal code: NSC; 0410462. ISSN: 0028-0836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198612
ED Entered STN: 19900302
Last Updated on STN: 19900302
Entered Medline: 19861204

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Tumour necrosis factors α and β inhibit virus replication and synergize with interferons

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Department of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

Tumour necrosis factor (TNF) and lymphotoxin were initially described as tumoricidal proteins that are produced by activated macrophages[1,2] and lymphocytes[3,4], respectively. Since TNF and lymphotoxin are structurally related, bind to the same cell surface receptor[5] and have indistinguishable biological activities[6,7], they have been designated as TNF-α and TNF-β, respectively[8]. The multiple activities[8-15] of these molecules indicate their importance in immunoregulative responses. Here we report that both TNF-α and TNF-β have antiviral activity and synergize with interferons (IFNs) in the induction of resistance to both RNA and DNA virus infection in diverse cell types. These effects of TNFs are not due to the induction of IFN synthesis. Virus-infected cells are selectively killed by TNFs and this activity is accelerated by IFN-γ. The production of TNFs is induced by viruses, further suggesting the importance of TNFs in the physiological antiviral response.

During an investigation of the effects of human TNFs on the production of IFN-γ by human lymphocytes, we observed that purified recombinant TNF-α and TNF-β had IFN-like activity and inhibited virus-mediated cytopathic effects. Pretreatment of human renal carcinoma cell line (7860) with TNF-α caused dramatic dose-dependent inhibition in viral replication, as determined by measuring virus yield after infection. These results were observed with both RNA viruses (encephalomyocarditis virus (EMCV) and vesicular stomatitis virus (VSV)) and DNA viruses (adenovirus-2 (Ad-2) and herpes simplex virus type II (HSV-2)) (Fig. 1). Similar results were found with TNF-β and no qualitative or quantitative difference between the two TNF types was observed (Fig. 1). Interestingly, the relative antiviral potencies of TNFs and IFN-γ varied with different viruses. TNF-α or TNF-β alone was able to inhibit VSV replication, whereas IFN-γ alone had no effect (Fig. 1*a*). TNFs were less potent (20–40-fold) than IFN-γ in protecting against EMCV (Fig. 1*b*) but were more potent (10–20-fold) in protecting against Ad-2 and HSV-2 (Fig. 1*c*, *d*). The antiviral activities of TNF-α and TNF-β were neutralized by the appropriate specific monoclonal antibodies but not by neutralizing antibodies specific for IFN-α, -β or -γ (Fig. 1*a*).

Antiviral effects of TNFs were observed with some but not all cell types. TNFs were also effective against VSV replication in the human cell lines RPMI-8226 (myeloma), and U87MG (glioblastoma), C127 mouse epithelial cells and Rat-1 fibroblasts. These results show that TNF-α and TNF-β have intrinsic

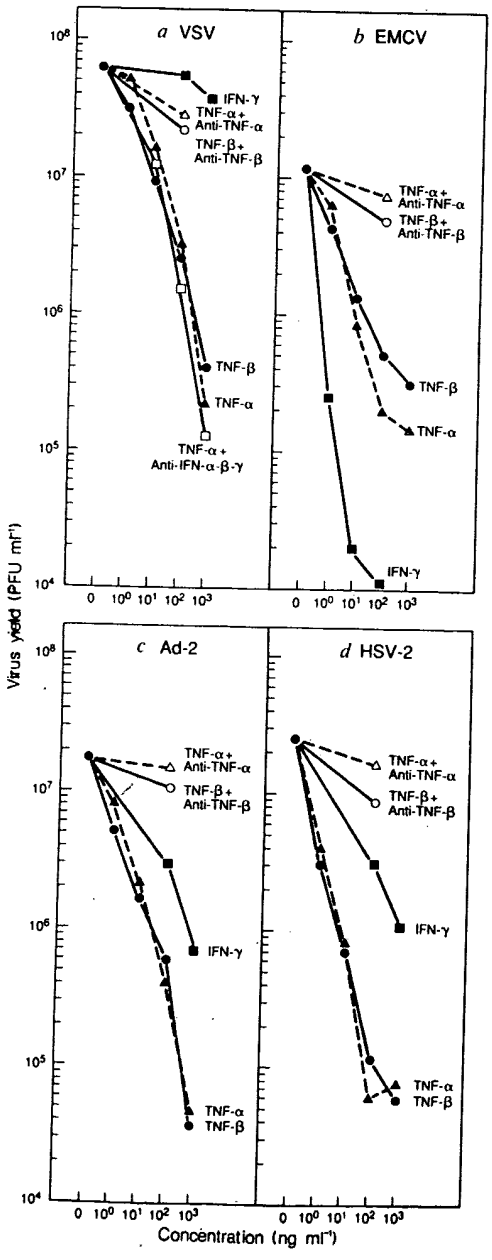

Fig. 1 TNF-α and TNF-β inhibit virus yield in 7860 cells. Human 7860 renal carcinoma cells (5×10^5 cells ml^{-1}) were seeded into 24-well tissue culture plates, treated with purified recombinant human TNF-α or TNF-β, at indicated concentrations or purified recombinant human IFN-γ in the presence or absence of antibodies. After 24 h incubation, the supernatants were removed before inoculation with VSV, EMCV, Ad-2 or HSV-2 viruses at multiplicities of infection (MOIs) of 10, 1, 100, and 100, respectively. After 2 h, the supernatants were aspirated to remove unadsorbed virus and the cells were incubated at 37 °C in medium containing 5% fetal bovine serum (FBS). After 24 h (VSV and EMCV) or 48 h (Ad-2 and HSV-2), the cultures were frozen and thawed twice to lyse the cells, centrifuged at 400*g* for 10 min and assayed for infectious virus yield in terms of plaque-forming units per ml (PFU ml^{-1})[27] on A549 cells. EMCV, Ad-2 and HSV-2 were propagated in A549 cells, while VSV was grown in BHK cells, and the viral titres were determined as described[27]. The VSV and EMCV virus stocks were purchased from the American Type Culture Collection. Ad-2 and HSV-2 were obtained from Dr K. Anderson (Genentech, Inc.). TNF-α or TNF-β at 1 μg ml^{-1} had undetectable toxic effects on uninfected 7860 cells. For neutralization experiments, 0.1 μg ml^{-1} of TNF-α or TNF-β was co-incubated with the corresponding anti-TNF and a mixture of anti-IFN-α, -β, -γ antibodies, respectively, for 4 h at 37 °C before adding to the cells. Polyclonal antibodies to IFN-α and -β were purchased from Boehringer and Lee Biomolecular Research Inc., whereas purified monoclonal antibodies against TNF-α, TNF-β and IFN-γ were generated at Genentech, Inc. The concentration of each anti-TNF monoclonal antibody used was 10-fold higher than that required to completely neutralize TNF cytotoxic activity. Similarly, the concentration of anti-IFN antibodies used was sufficient to neutralize a 10-fold excess of the homologous IFNs. The specific activities for purified recombinant human TNF-α and TNF-β were 3×10^7–4×10^7 units mg^{-1} determined in a standard cytotoxic assay using LM cells[28]. The specific activities for purified recombinant human IFN-αA, IFN-β and IFN-γ were 2×10^8 units mg^{-1}, 3×10^7 units mg^{-1} and 4×10^7 units mg^{-1}, respectively, determined in a standard cytopathic effect inhibition assay using human A549 cells challenged with EMCV.

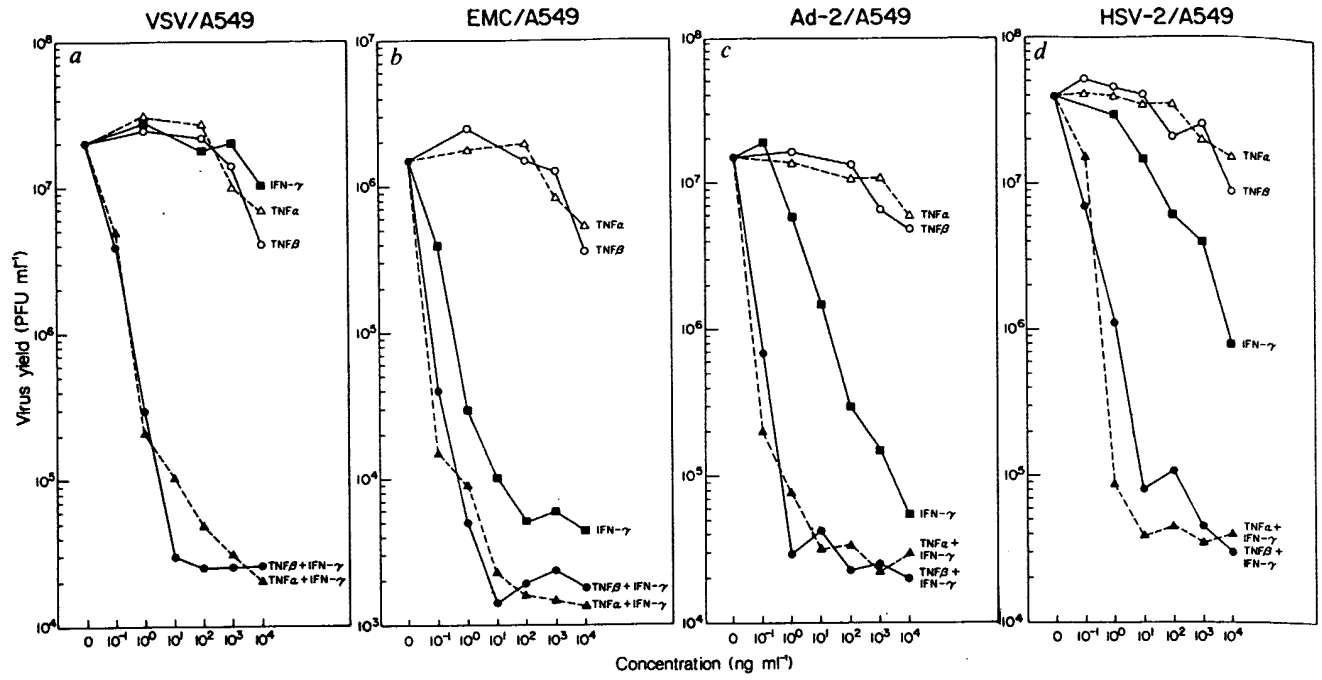

Fig. 2 TNFs enhance the ability of IFN-γ to inhibit virus replication. Confluent A549 cells were treated with the TNF-α, TNF-β, IFN-γ or the indicated concentrations for 18 h before challenge with VSV (*a*), EMCV (*b*), Ad-2 (*c*) or HSV-2 (*d*) at MOIs of 10, 1, 100 and 100, respectively. The inhibition of virus yield was determined as described in Fig. 1.

antiviral activity and can induce an IFN-like cellular state of viral resistance.

Since IFNs have been shown to enhance the cytotoxic activity of TNFs[16,17], we examined the effect of TNFs on the antiviral activity of IFNs. IFNs protect against VSV infection in most cells[18], but purified recombinant human IFN-γ has little anti-VSV activity when assayed on the A549 human lung carcinoma cell line. However, in the presence of purified recombinant human TNF-α or TNF-β, IFN-γ was able to protect A549 cells from VSV-mediated cytopathic effects (data not shown). Unlike IFN-γ, IFN-αA and IFN-β alone have some anti-VSV activity on A549 cells. This activity was also enhanced by TNF-α, although the total anti-VSV activity was no greater than that seen with TNF-α and IFN-γ (data not shown). Thus, the antiviral enhancing activity of TNFs is not restricted to IFN-γ.

The antiviral enhancing activity of TNFs can also be observed by measuring reduction in virus yield. Neither TNF nor IFN-γ alone inhibited VSV replication in A549 cells (Fig. 2*a*). However, TNF in combination with IFN-γ caused a large reduction in virus yield (about 2,000-fold greater than observed for IFN-γ alone). A 99% reduction required only 1 ng ml^{-1} of each cytokine in combination. Furthermore, TNF-α and TNF-β were able to enhance the activity of IFN-γ against EMCV in A549 cells, especially at low concentrations of IFN-γ (Fig. 2*b*).

The synergistic antiviral effect of TNF-α or TNF-β is not restricted to RNA viruses but is also observed with DNA viruses such as Ad-2, Ad-5, HSV-1 and HSV-2. The combination of TNF-α or TNF-β with IFN-γ strongly inhibited replication of Ad-2 and HSV-2 in A549 cells (Fig. 2*c* and *d*). At concentrations higher than those required for protection against EMCV, IFN-γ

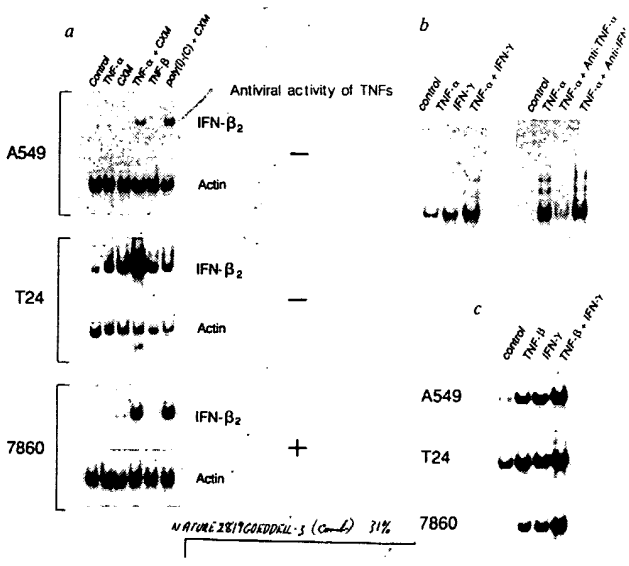

Fig. 3 Regulation of 'IFN-β_2' mRNA and induction of 2-5A synthetase mRNA by TNFs. *a*, A549, T24 and 7860, cells were incubated with TNF-α (0.1 μg ml^{-1}), TNF-β (0.1 μg ml^{-1}), cycloheximide (CXM) (1 μg ml^{-1}), poly(I)·poly(C) (10 μg ml^{-1}), or the indicated combinations. Total cytoplasmic RNA was extracted[29] after 4 h exposure to TNFs. Poly(A) RNA was prepared by oligo(dT) cellulose[30]. Electrophoresis and Northern hybridization using 0.7 μg RNA per lane, were performed as described[31]. ^{32}P-labelled 'IFN-β_2' was used as hybridization probe. The probe was prepared using DNA polymerase I (Klenow fragment) to fill in the 3′ ends of overlapping synthetic 45- and 42-mers and corresponds to nucleotides 32 to 55 of the 'IFN-β_2' sequence[32]. As a control, the same RNA filter was also hybridized with ^{32}P-labelled α-actin (ref. 33) cDNA probe to indicate that the levels of mRNA on each lane were similar. *b*, TNF-α induces the expression of 2-5A synthetase mRNA. Confluent A549 cells treated with TNF-α (0.1 μg ml^{-1}), IFN-γ (0.1 μg ml^{-1}) or the combination; treated with TNF (0.1 μg ml^{-1}) with or without excess monoclonal anti-TNF-α or a mixture of a 10-fold excess polyclonal antibodies against IFN-α, -β or -γ. *c*, Confluent A549, T24 and 7860 cells treated with TNF-β (0.1 μg ml^{-1}) or IFN-γ (0.1 μg ml^{-1}) or the combination. Total cytoplasmic RNA was extracted. Poly(A) RNA was prepared and Northern hybridization was performed after 12 h treatment. Poly(A) RNA (~1 μg per lane) was hybridized with ^{32}P-labelled 2-5A synthetase probe[34], which was prepared by filling in at the 3′ end of two overlapping synthetic 45- and 42-mers corresponding to nucleotides 331 to 395 of the 2-5A synthetase cDNA sequence[34].

lone provided partial protection against Ad-2 and HSV-2, whereas TNF-α or -β alone did not. In the presence of low concentrations of IFN-γ (1 ng ml^{-1}), TNF concentrations as low as 1 ng ml^{-1} were sufficient to inhibit Ad-2 and HSV-2 virus yield (Fig. 2*c* and *d*). Thus, TNFs and IFN-γ together are more efficient in inhibiting both RNA and DNA virus yield than either molecule alone.

Various cell lines were tested for their ability to respond to the antiviral potentiating activity of TNFs. The antiviral enhancing activity of human TNFs was also observed on human (HT-1080, lung fibrosarcoma; HeLa cervical carcinoma; T24 bladder carcinoma; HT-29 colon carcinoma; 7860 renal carcinoma; ST-486 Burkitt's lymphoma; RPMI-8226 myeloma and U87MG glioblastoma) and non-human (MDBK bovine kidney fibroblast; Rat-1 rat fibroblast; C127 mouse epithelial cells and Raw-264 mouse macrophage) cell lines. The combination of IFN-γ and TNF-α resulted in inhibition of VSV replication from 50 to 4,000 times greater than IFN-γ alone in these cells. Thus, the antiviral potentiating activity of TNFs, as with their tumoricidal activity, appears to be species-nonspecific.

Three different experimental approaches indicated that the antiviral activities of TNF are not caused by the induction of IFNs. First, concentrated medium from cells pretreated with TNF contained no detectable antiviral activity. Second, antibodies specific for IFN-α, -β and -γ could not neutralize the anti-VSV activity of TNF-α in 7860 cells (Fig. 1*a*). Third, Northern hybridization of RNA from TNF-treated A549 cells with specific IFN-α[19], IFN-β[20] and IFN-γ[21] complementary DNA (cDNA) probes failed to detect any of these mRNAs.

Recently, it was suggested that the action of TNF-α may be mediated through the induction of another molecule that has been termed 'IFN-β_2'[22]. The mRNA for 'IFN-β_2' can be induced by cycloheximide (CXM)[22–24], but whether or not the 'IFN-β_2' translation product has antiviral activity remains controversial[25]. Pretreatment of T24 cells with CXM for 2 h induced high levels of mRNA for 'IFN-β_2' (Fig. 3*a*) but resulted in no detectable antiviral protection. TNFs induced 'IFN-β_2' mRNA in T24 but not in 7860 or A549 cells (Fig. 3*a*), although they have antiviral activity in 7860 cells but not in T24 or A549 cells. The induction of 'IFN-β_2' mRNA was greater when cells were treated with both TNF-α and CXM than either alone (Fig. 3*a*). However, this treatment for 2 h followed by removal of TNF and CXM, and further incubation for 12 to 24 h, failed to protect against EMCV or VSV infection in T24 or A549 cells. These results show that there is no correlation between the antiviral activity of TNFs and the induction of 'IFN-β_2' mRNA. These experiments demonstrate that the antiviral activities of TNFs are direct and are not mediated through the induction of IFNs.

The synthesis of the enzyme 2′,5′-oligoadenylate synthetase (2-5A synthetase) is strongly induced by all IFNs, making it a good marker of IFN activity[26]. TNF-α, like IFNs, induces the expression of 2-5A synthetase mRNA in A549 cells (Fig. 3*b*). The mRNA is first detectable 4 h after induction by TNF-α, or IFN-γ, or the combination, and reaches its maximal level at 12 h. The induction of 2-5A synthetase mRNA by TNF-α was neutralized by monoclonal antibodies directed against TNF-α but not by antibodies against IFN-α, -β or -γ (Fig. 3*b*). This result further demonstrates that induction of 2-5A synthetase mRNA by TNF-α is not mediated through the production of IFNs. TNF-β can also induce 2-5A synthetase mRNA (Fig. 3*c*). The combination of TNFs and IFN-γ gave stronger induction than either cytokine alone (Fig. 3). Thus, TNFs share with IFNs the ability to induce 2-5A synthetase. However, induction of 2-5A synthetase alone is not sufficient to mediate antiviral protection in these cells because neither TNF nor IFN-γ alone protects against VSV in A549 cells, yet either alone induces significant levels of 2-5A synthetase mRNA.

In addition to enhancing the induction of an antiviral state in uninfected cells, TNFs are also able selectively to kill virus-infected cells. Under normal growth conditions in the absence

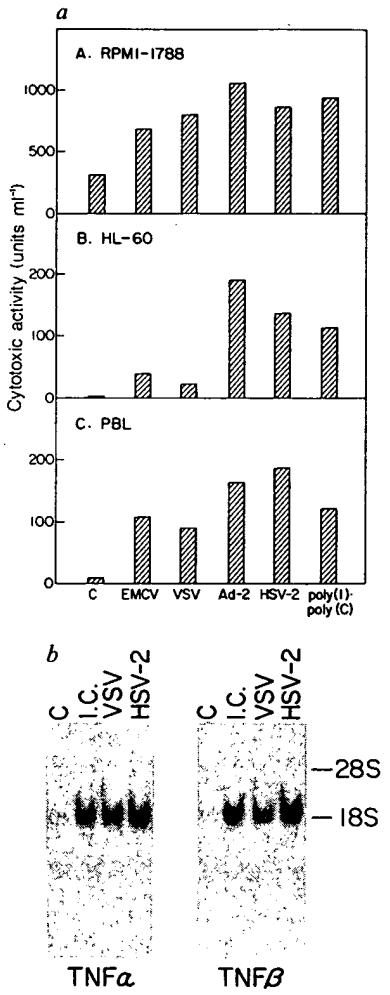

Fig. 4 Viruses and poly(I)·poly(C) induce TNF production. *a*, The human B lymphoblastoid TNF-β producing cell line RPMI-1788 (ref. 7) (A) and the TNF-α-producing cell line HL-60 (ref. 6) (B), and human peripheral blood leukocytes (C) isolated from Ficoll-hypaque gradients at a concentration of about 1×10^6 cells ml^{-1} were incubated with EMCV, VSV, Ad-2 or HSV-2, at MOIs of 1, 10, 100 and 100, respectively, or poly(I)·poly(C) (10 μg ml^{-1}) for 2 h, washed and further incubated in medium at 37 °C. After 5 h, supernatants were collected and assayed for cytotoxic activity[28]. *b*, Northern hybridization of RNA derived from human peripheral blood leukocytes (2×10^6 cells ml^{-1}) induced with poly(I)·poly(C) (10 μg ml^{-1}), VSV, or HSV-2 at MOIs of 10 and 100, respectively. RNAs extracted after 4 and 12 h infection were hybridized with ^{32}P-labelled TNF-α and TNF-β cDNA probes, respectively, which were ^{32}P-labelled *Ava*I-*Hin*dIII restriction fragment (578 bp) from human TNF cDNA[6] and an *Eco*RI restriction fragment (~650 bp) from the coding region of human TNF-β cDNA[7], respectively.

of virus infection, A549 cells are resistant to the cytotoxic activity of TNFs at 24 h even in the presence of IFN-γ. The viability of VSV-infected cells 12 and 18 h after virus infection was 92% and 70%, respectively, whereas the viability of TNF-treated, virus-infected cells had dropped to 30–40%. Similarly, TNFs selectively killed Ad-5-infected A549 cells (data not shown). Thus, virus-infected cells are more susceptible to lysis by TNFs than are uninfected cells, and lyse earlier than they would have in the absence of TNFs.

Although IFN-γ (0.1 μg ml^{-1}) alone did not selectively kill VSV-infected A549 cells, it accelerated the lysis of virus-infected cells by TNFs. Within 18 h, the combination of IFN-γ and TNFs had killed virtually all VSV-infected cells. An increase in vulnerability of virus-infected cells to killing by TNFs was also found with other cell lines, such as 7860 renal carcinoma and

U87MG glioblastoma cells. These results suggest that the selective killing by TNFs is a general phenomenon and not restricted to a specific cell type or virus.

Because TNFs potentiate the antiviral activity of IFNs, it was of interest to determine whether viruses could induce the production of TNFs. When the human B lymphoblastoid cell line RPMI-1788 (known to produce TNF-β[7]) and promyelomonocytic leukaemia cell line HL-60 (known to produce TNF-α[6]) were challenged with EMCV, VSV, Ad-2, or HSV-2, significant levels of TNFs were produced (Fig. 4*a*). Poly(I)·poly(C), an inducer of IFN-α and IFN-β synthesis, also stimulated the production of TNFs in these cells. Normal peripheral blood leukocytes also produced significant levels of TNF activity after exposure to these inducers (Fig. 4*a*). Northern hybridization of poly(A) RNA from leukocytes with TNF-α and TNF-β cDNA probes showed that mRNA for both these genes were induced by this treatment (Fig. 4*b*).

Our results show that TNF-α and TNF-β have intrinsic antiviral activity on certain cells and dramatically synergize with IFNs in terms of antiviral activities on diverse cell types. The mechanisms by which TNFs alone specifically induce an antiviral effect in certain cells is not known. However, cells (such as T24) that are not protected by TNFs do have TNF receptors[5,9] and also respond to TNFs as measured by induction of 2-5A synthetase and 'IFN-β_2' mRNA (Fig. 3).

The different spectra of antiviral activity of TNFs and IFN-γ, together with their synergistic actions, suggest that TNFs and IFN-γ exert their antiviral effects through at least partially independent mechanisms. The synergistic action of TNFs and IFN-γ is not due to induction of IFN-γ receptors because TNFs have no effect on the expression of IFN-γ receptors (Ramani Aiyer, personal communication). Further work is required to determine the mechanism of TNF-induced antiviral activity and its synergy with IFNs.

The data presented here suggest that a major function of TNFs may be to combat viral infection by at least two mechanisms: by inducing resistance in uninfected cells, and by selectively killing virus-infected cells in order to prevent viral spread. The production of TNFs and IFNs in response to viral infection, and their positive cooperation with each other, suggest that together they form a powerful and general host defence system. The potency and effectiveness of these molecules in combination holds promise for the future treatment of viral diseases.

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An activated Harvey *ras* oncogene produces benign tumours on mouse epidermal tissue

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Studies of the mutagenic action required for specific chemicals to produce benign or malignant tumours suggest that in mouse skin at least two genetic events occur before carcinoma formation[1]. The isolation of an activated form of the c-*ras*^{H} gene from skin papillomas has provided evidence that this gene may be a target for the first mutation, which could constitute the initiating mutation in skin carcinogenesis[2,3]. *In vitro* studies indicate that the v-*ras*^{H} gene of Harvey murine sarcoma virus (Ha-MSV), a replication-defective transforming retrovirus, could impart a conditional initiated phenotype on cultured keratinocytes by blocking their ability to differentiate terminally and arresting them in a late basal cell stage of maturation[4]. We now show that when the Ha-MSV v-*ras*^{H} gene is introduced into cultured keratinocytes by a defective retroviral vector, skin grafts constructed with cells carrying the mutated *ras* oncogene produce papillomas on athymic nude mouse recipients. Furthermore, the expression of the exogenous oncogene seems to be regulated at the transcriptional level in the differentiated portions of the benign tumour.

Mouse keratinocytes were cultured in medium containing 0.05 mM Ca^{2+}, a condition which selects for basal cells[5]. A variant Ha-MSV genome (see Fig. 1) was used to generate a virion preparation devoid of helper virus genomes by transfection of the cloned Ha-MSV DNA into $\psi 2$ cells, which contain a packaging-defective Moloney murine leukaemia virus (Mo-MLV) genome as described by Mann *et al.*[6]. Morphological transformants were selectively cloned and supernatants from cloned cell lines assayed for transforming virus production by *in vitro* focal transformation of NIH 3T3 cells. Three days after seeding, epidermal cells were exposed to $\psi 2$ cell supernatants containing $\sim 10^6$ Ha-MSV focus-forming units (determined on NIH 3T3 cells); the non-productively infected cells behaved identically to cells productively infected with typical mixtures of Ha-MSV and helper virus particles in culture medium containing either 0.05 mM Ca^{2+} (increased proliferation) or 1.4 mM Ca^{2+} (blocked differentiation)[4,7]. Unlike cells that were productively transformed, conditioned medium from keratinocyte cultures infected with the $\psi 2$ preparation did not transform NIH 3T3 cells.

Non-productively infected keratinocytes were removed from culture within 5 days of infection, combined with freshly isolated dermal fibroblasts[8] and grafted onto prepared skin sites on nude

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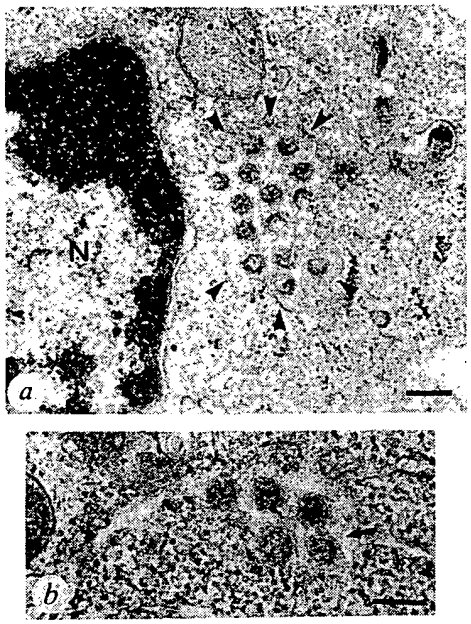

Fig. 5 Viral particles in cultured KD PBMC. *a*, Mononuclear cell containing; arrowheads, intracellular vacuole with many uniformly sized viral particles (105–118 nm), N, nucleus, (magnification ×47,500, enlargement ×2.5) scale bar = 200 nm. *b*, Intracellular vacuole at higher magnification (×66,500, enlargement ×3.5). Arrow, viral particle budding from the vacuole membrane. Scale bar = 200 nm. KD PBMC were cultured for 7 days in IL-2-containing medium (Fig. 1 legend). Cell pellets were fixed in formaldehyde-glutaraldehyde for one hour, washed and post-fixed in 1.3% osmium tetroxide in 0.1 M s-Collidine buffer, *p*H 7.3. The cells were stained with 3% uranyl acetate in 75% ethanol and embedded in epon. Thin sections were then stained with lead citrate and examined in a Philips 301 electron microscope.

lular vacuoles (Fig. 5*a*). The particles are 105–118 nm in diameter, have an electron-dense core and an outer membrane and some appear to bud from the vacuole membrane (Fig. 5*b*). Similar viral particles were not observed in cultured PBMC from 5 control subjects. These preliminary observations support the possibility that the polymerase activity found in PBMC cultures of KD patients is associated with a virus.

Although the human retroviral illnesses caused by HTLV-I, -II and HIV are usually thought of as chronic syndromes, acute infection with HIV has been reported to be associated with a self-limited illness characterized by fever, rash, lymphadenopathy and mucositis[11]. In contrast with most HTLV patients, the immune systems of KD patients return to normal after the acute illness is over. Further studies are needed to elucidate the possible importance of lymphocytotropic viruses in the pathogenesis of Kawasaki disease.

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Table 1 Characterization of polymerase activity

Patient lymphocyte culture		Picomoles of dTMP incorporated: Complete reaction	Heat inactivation*	−Template & primer†	−Mg^{2+} +EDTA‡
C.N.	10 days	97.8	0	0.6	—
J.J./HUT-78	12 days	7.4	—	—	0.1
A.B./HUT-78	7 days	9.8	0	—	0.1
C.N./HUT-78	11 days	7.1	—	0.3	—
R.G./HUT-78	16 days	16.0	—	5.5	—
HUT/78		1.5	0.6	0.9	1.2
Control		0.3	0	0.4	0
Control/HUT-78 6 days		1.9	1.1	0.2	0.4
MT-2 (HTLV-I cell line)		55.4	0	0.7	0
Buffer A		1.9	0.2	0.3	0.5

See Fig. 1 legend for reaction conditions.
* Heat inactivation of pellet was for 10 min at 80 °C before addition of reaction mixture.
† Template & primer (poly rA:oligo dT) omitted.
‡ $MgCl_2$ omitted, 1 mM EDTA (final concentration) added.

Antiviral effects of recombinant tumour necrosis factor *in vitro*

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Tumour necrosis factor (TNF) was first described[1] as a factor in the serum of mice injected with tubercle bacilli (BCG) and several days later with lipopolysaccharide (LPS). The gene encoding TNF has recently been cloned and pure recombinant human TNF is now available[2,3]. TNF is known for its *in vivo* antitumour effect and *in vitro* cytotoxicity on certain transformed cell lines[4,5]. Similarities in amino acid sequence and biological activity to lymphotoxin and cachectin have been reported[2,6], and very recently a growth-factor-like activity on diploid fibroblasts was observed[7]. There is no similarity between these proteins and interferons (IFNs), which are also induced during *in vivo* induction of TNF[8]. Here we describe the antiviral activity of pure recombinant human TNF in a typical *in vitro* antiviral assay which we discovered while investigating the possible role of TNF as an inducer of IFN.

Human laryngeal carcinoma cells (HEp-2) were grown in 96-well plates for 48 h. When cells had reached confluency, the medium was decanted and vesicular stomatitis virus (VSV) added at a multiplicity of infection (MOI) of 0.04. After 32 h, plates were fixed and stained. In the absence of TNF, we observed complete destruction of the cell monolayer, but pretreatment with TNF leads to partial or complete protection. Concentrations as low as 1 ng ml^{-1} inhibit the development of the virus-induced cytopathic effect (Table 1). As a control, we used IFNs as the effects of TNF are indistinguishable from those of IFNs. Using MOIs of VSV > 1, preincubation with TNF for at least 4 h was necessary to achieve protection. At a lower MOI of 0.04 it was even possible to partially protect the cells

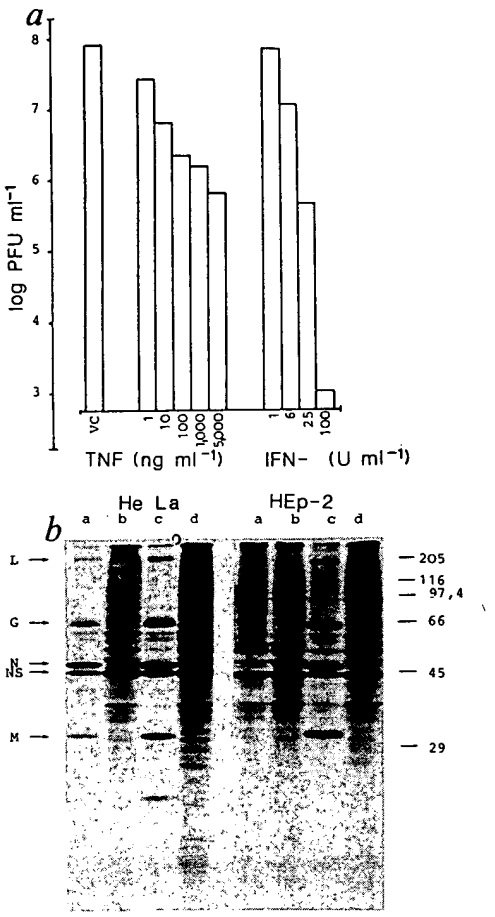

Fig. 1 Effect of TNF on virus yield (*a*) and viral protein synthesis (*b*). *a*, Virus yield reduction assay. HEp-2 cells, grown to confluency in microtitre plates, were overlayed with medium (MEM, 10% calf serum, CS) containing different doses of TNF or IFN for 20 h. Subsequently, cells were washed and infected with VSV in modified Eagle's medium (MEM) at a MOI of 0.04. After 1 h, the cell monolayers were washed twice and 200 μl fresh MEM, 5% CS per well was added. (Incubation at 37 °C, 5% CO_2.) Cells were frozen 30 h post-infection at −70 °C. A plaque assay for determination of virus yields was performed by titration on confluent RITA-cell monolayers in 6-well plates. After freeze-thawing, supernatants containing virus were diluted with basal Eagle's medium (BEM) in 10-fold dilution steps and RITA-cell monolayers were infected with 200 μl virus suspension per well. After infection (1 h) overlay medium (BEM, 1% carboxymethyl cellulose, 5% fetal calf serum) was added. Later (24 h) cells were fixed with 3% formaldehyde and stained with gentian violet and the plaques counted to determine plaque-forming units (PFU) ml^{-1}. *b*, Inhibition of the synthesis of viral proteins by TNF in HEp-2 or HeLa cells. Cells were grown to confluency in 24-well plates and incubated with either human IFN-β (500 U ml^{-1}) or TNF (5 μg ml^{-1}) for 20 h followed by infection with VSV at a MOI of 10. After viral infection (4 h) medium was removed and replaced by methionine-free BEM containing ^{35}S-methionine at 35 μCi ml^{-1} (150 μl per well). The cells were pulse-labelled for 2 h, washed and dissolved in 100 μl per well sample buffer for SDS-PAGE. Electrophoresis was performed in 12% gels according to Laemmli[11] (40 μl of each sample). Gels were stained with Coomassie brilliant blue to visualize marker proteins and dried after treatment with enhancer (EN^{3}HANCE, NEN). Autoradiographs of gels are shown. Lane *a*, TNF-treated, virus-infected cells; *b*, IFN-treated, virus-infected cells; *c*, virus control; *d*, cell control. Relative molecular mass (M_r) marker proteins (×1,000) SDS-6H high standard mixture (Sigma); VSV proteins: RNA-dependent RNA polymerase (L); glycoprotein (G); nucleoproteins (N, NS); nonglycosylated membrane protein (M).

Table 1 Cell protection assay showing the antiviral effect of TNF

TNF (ng ml^{-1})	HEL*	HEp-2*	HEp-2†
	Per cent of intact cell monolayer		
1,000	100	100	ND
300	100	100	100
100	100	100	100
30	100	100	100
10	90	100	100
3	80	80	100
1	40	40	100
0.3	10	20	50
0.1	0	0	10

Cells were grown to confluency in microtitre plates. After treatment for 20 h with different concentrations of TNF batch I(*) or TNF batch II(†), overlaying medium was removed and VSV added at a MOI of 0.04 in 0.01 ml of serum-free medium. After infection (1 h) 0.1 ml complete medium was added. The protective effect of TNF was expressed as the extent of viable cell monolayers estimated after staining with gentian violet 32 h after infection and compared with an untreated, uninfected cell control (100%). The monolayers of untreated and infected cells (virus control) were completely destroyed (0%) under these conditions. TNF was produced by recombinant DNA methods and purified to homogeneity from bacteria as described previously[2,3]. It was homogeneous by the criteria of SDS-polyacrylamide gel electrophoresis (PAGE)[11] and reverse-phase HPLC. Biological activity was measured by the L929 test[5] and endotoxin content estimated by the LAL test[12]. Two different batches of purified TNF were used: batch I, biological activity, 1×10^7 U mg^{-1} protein; endotoxin content, >0.6, <3.0 ng mg^{-1} protein. Batch II, biological activity, 1.5×10^7 U mg^{-1} protein; endotoxin content, >0.069, <0,137 ng mg^{-1} protein.

by adding TNF as late as 7 h after virus infection (data not shown). This suggests that TNF only protects cells that are not yet infected.

The antiviral effect of TNF is not limited to VSV but can be demonstrated with two additional viruses, encephalo-myocarditis virus (EMCV) and herpes simplex virus (HSV). TNF not only prevents the development of the cytopathic effect but in cells pretreated with different doses, virus yield is reduced in a dose-dependent manner (Fig. 1*a*). Like interferon, TNF intervenes at an early stage of viral replication as shown by the complete inhibition of the formation of viral proteins in HEp-2 cells pretreated with TNF or IFN (Fig. 1*b*). So far, we have demonstrated the antiviral effect of TNF with three out of six cell lines of human origin and with one out of four cell lines of animal origin: HEp-2, human embryonic lung fibroblasts (HEL), human embryonic lung fibroblasts (WI-38) and mouse embryonic fibroblasts (MEF) are susceptible to the antiviral effect of TNF, whereas human cervix carcinoma cells (HeLa), human skin fibroblasts (BUD-8) and human amnion cells (WISH) as well as mouse transformed fibroblasts (L929), rat embryonic fibroblasts (REF) and monkey kidney cells (RITA) are not protected against viral infection by pretreatment. Susceptibility to the antiviral activity was not restricted to cells with transformed phenotype. There is no indication that treatment with doses of TNF active in antiviral protection (up to 3 μg ml^{-1}) have any negative effect on cell viability of uninfected Hep-2 cells. Neither cell growth nor plating efficiency is affected by the TNF doses used. However, when assayed for the susceptibility to the toxic effect of TNF in the presence of actinomycin D, HEp-2 cells show similar sensitivity to L929 cells (data not shown). Yet the latter are not protected against viral infection with VSV by pretreatment with TNF alone, suggesting that the antiviral and the cytotoxic effects of TNF are separate activities.

In experiments using antibodies capable of inhibiting the activities of human IFNs, we found that anti-IFN-α or anti-IFN-γ added to HEp-2 cells simultaneously with TNF does not affect the TNF-mediated antiviral protection. Anti-IFN-β results in a partial reduction of this effect. However, it is not possible to neutralize completely the antiviral effect of TNF even with high

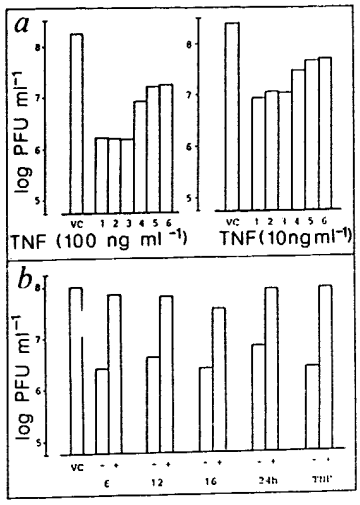

Fig. 2 Reversal of TNF-induced antiviral activity. *a*, Neutralization of the TNF antiviral effect by anti-human IFN antibodies; *b*, antiviral activities in the supernatants of TNF-treated HEp-2 cells.

Methods. *a*, TNF was mixed with anti-human IFN antibodies (previously confirmed to inhibit the antiviral activities of interferons) to final concentrations of 100 ng ml^{-1} TNF or 10 ng ml^{-1} TNF in MEM, 10% CS. Final concentrations of polyclonal antibodies (Paesel, Frankfurt) expressed in neutralizing units (NU) per ml were: (1) 0, TNF control; (2) 500 NU ml^{-1}, anti-IFN-α; (3) 500 NU ml^{-1}, anti-IFN-γ; (4) 50 NU ml^{-1}, anti-IFN-β; (5) 500 NU ml^{-1}, anti-IFN-β; and (6) 2,500 NU ml^{-1}, anti-IFN-β. Triplicates of each of these mixtures were added to confluent HEp-2 cells in microtitre plates and incubated at 37 °C (5% CO_2). Cells were then infected with VSV and virus yields were determined with the plaque assay described in Fig. 1*a*. VC, Virus control. *b*, HEp-2 cells grown to confluency in microtitre plates were incubated with 100 µl per well MEM, 10% CS containing 200 ng ml^{-1} TNF. After 6, 12, 16 and 24 h incubation, supernatants of 10 wells were collected. One aliquot of each supernatant (−) was mixed with an equal volume of medium (MEM, 10% CS), another aliquot (+) with an equal volume of medium containing 20 µg ml^{-1} monospecific anti-TNF antibody (BASF) to neutralize the activity of residual TNF. (10 µg ml^{-1} anti-TNF antibody completely neutralizes the antiviral activity of 100 ng ml^{-1} TNF as shown in the two right-hand columns.) After 2 h at room temperature these mixtures were added to fresh HEp-2 cells in microtitre plates (in triplicate) and incubated for 20 h (37 °C, 5% CO_2). Infection with VSV and determination of virus yields was performed as described in Fig. 1*a*.

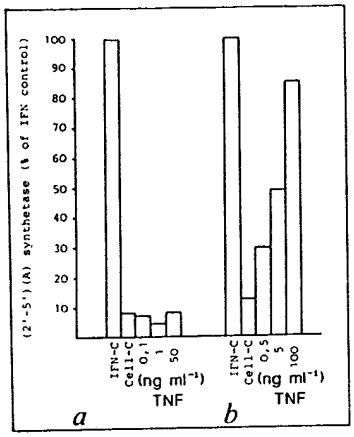

Fig. 3 Assay for IFN-induced (2′–5′)(A)$_n$ synthetase in HEp-2 cells. Assay for induction of (2′–5′) (A) synthetase by TNF and IFN was performed as described elsewhere[13]. Briefly, cells were grown in 14-cm Petri dishes to ~80–90% confluency (*a*) or to full confluency (*b*) and incubated with human IFN (300 U ml^{-1}) or different concentrations of TNF (batch I) for 20 h. After collecting and washing, the cells were lysed in Nonidet P-40-containing lysis buffer and centrifuged at 12,000*g* for 10 min at 4 °C. Protein content of the supernatants was determined with the Bio-Rad protein assay kit[14] with bovine serum albumin as protein standard. Protein (200 µg) from each supernatant was added to 20 µl poly(IC)–agarose (P.L. Biochemicals) followed by incubation for 1 h at room temperature and three washes in lysis buffer to remove unbound material. Assay mix (15 µl) containing 5 mM ATP (25 µCi [α-^{32}P]ATP per µmol ATP) was added and incubated for 6 h at 30 °C. Subsequently the poly(IC)–agarose was removed by centrifugation and 15 µl supernatant treated with alkaline phosphatase. Inorganic phosphate was adsorbed on alumina by passing the samples through small alumina columns under acidic conditions (*p*H 2). The amount of oligo (2′–5′)(A)$_n$ (^{32}P-labelled) was evaluated by determining the c.p.m. in the eluates in a liquid scintillation counter.

doses of anti-IFN-β (Fig. 2*a*). Most of the antiviral activity of supernatants from TNF-treated HEp-2 cells can be neutralized by anti-TNF antibodies (Fig. 2*b*), suggesting that there is only very little IFN present in the supernatants. The maximal remaining antiviral activity observed led to 0.5 log virus yield reduction, comparable to the effect of 2–3 U ml^{-1} of external IFN-β added to HEp-2 cells before infection with VSV.

We next demonstrated that the interferon-induced enzyme (2′–5′)(A)$_n$ synthetase can be induced in HEp-2 cells grown to full confluency in a dose-dependent manner by treatment with doses active in antiviral protection. In cells 80–90% confluent the (2′–5′)(A)$_n$ synthetase level is not enhanced by treatment with TNF (Fig. 3), in agreement with an experiment where TNF-induced antiviral activity is compared in HEp-2 cells at different states of confluency and showing that fully confluent cells are most susceptible to the antiviral effect of TNF (data not shown). Induction of this enzyme can be regarded as an indication of the presence of interferon. However, using Northern blot techniques and specific probes for human IFN-α and IFN-β genes, we failed to detect any IFN-specific RNA in HEp-2 cells treated with various doses of TNF (data not shown).

After submitting our manuscript we became aware of data of the group of Jan Vilcek showing the induction of IFN-β_2 by TNF in serum-starved human foreskin fibroblasts leading to inhibition of EMCV replication in these cells. As anti-human IFN-β antibodies can neutralize this effect they concluded that IFN-β_2 is the mediator of the antiviral activity of TNF[9].

Our data (Fig. 2) only partially agree with this conclusion. Although TNF seems to be a weak inducer of IFN-β-like activity, the amount of the detectable IFN in the supernatants of TNF-treated HEp-2 cells is not sufficient to account for the observed virus yield reductions in these cells. Therefore, we propose that the antiviral effect of TNF is not exclusively mediated by the induction of IFN but that there is an additional mechanism caused by the action of TNF itself. Both TNF and the TNF-induced interferon may act together in establishing the antiviral state of the cells.

The mechanisms of TNF-induced antiviral protection need to be explored further to determine whether they are similar to the known mechanisms of antiviral activity of interferon. Note that TNF appears to be the only lymphokine apart from interferon that protects cells against viral infection. Old[10] recently suggested that the physiological role of TNF is that of a protective agent against infectious diseases and this view is supported by our present findings.

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